Temporal Denitrification at the Landscape Scale in a Black Soil

C. van Kessel, D.J. Pennock, and R.E. Farrell
Dept. of Soil Science, Univ. of Saskatchewan, Saskatoon, SK, S7N 0W0

ABSTRACT

Landscape scale and seasonal pattern of denitrification activity have to be incorporated in a model to estimate total N losses. A study was conducted to exam the seasonal variability of denitrification in a landscape near Blaine Lake, Saskatchewan. A 120-m by 120-m sampling grid, separated by a spacing of 10 m, was established in a Black Chernozem soil. The area was surveyed, landform elements identified and from each landform element ten sampling points were further selected and sampled throughout the season for denitrification activity by the acetylene-blockage approach. Soils samples were taken seven times during the entire 1991 season before the area was prepared for seeding in the spring, following precipitation events during the growing season , and in the fall at the onset of frost. Following incubation, soil samples were analyzed for percent moisture, NH_4^+ and NO_3^-, soluble organic carbons, and total soil respiration. The distribution of denitrification activities were highly skewed and followed a distinct landscape pattern that remained consistent throughout the year. Denitrification activity increased significantly after the occurrence of a precipitation event and was further enhanced after the application of fertilizer-N, ceased toward the end of the growing season and became zero at the fall sampling. Moisture was the most dominant parameter controlling denitrification activity followed by the concentration of and NH_4^+ and NO_3^-. The highest denitrification activity occurred on the divergent and convergent footslopes, the lowest activity on the divergent shoulder and upper level landform elements, a landscape scale pattern that remained consistent throughout the year, independent of the magnitude of activity. Ambient evolution of N_2O and denitrification activity followed predominantly a similar temporal and landscape scale pattern. By estimating the duration of a denitrification following a precipitation event at the various landform elements and correcting for the percentage each landform element occupies in the landscape, the total denitrification per precipitation during the early part of the season was estimated at 357 g N ha^{-1} $cycle^{-1}$. In conclusion, results indicates that landscape scale pattern of denitrification remained constant throughout the growing season and was predominantly induced by precipitation events.

INTRODUCTION

Denitrification is predominantly an anaerobic biological process that convert NO_3^- into N_2O and N_2. The conversion is often carried out by soil organism that use O_2 under aerobic conditions as an electron acceptor but have the capability to use NO_3^- as an acceptor under low levels of soil O_2 pressure. Whereas the production of N_2 does not pose any environmental repercussions, the production of N_2O has been shown to be one of the green house gasses and contributes to the destruction of the ozone layer. The partitioning of the gaseous products, N_2 and N_2O, is dependent on various factors such as pH of the soil, the availability of organic carbon sources, the concentration of NO_3^-, and the O_2 pressure in the soil (Firestone, 1982).

Denitrification activity under field conditions has been found to be variable and the frequency of its activity is often log normally distributed (Parkin et al., 1987). A large percentage of soil cores analyzed for denitrification activity often show low levels of activities whereas a small percentage will show exceptional high rates of denitrification, located in so-called hot spots (Parkin, 1987). The apparent high variability of *in situ* denitrification activity would make a seasonal estimate of denitrification in an agriculturally cultivated field hazardous.

Various parameters which are reported to control denitrification, i.e. soil moisture content and the concentration in NO_3^-, were found to explain less than 50% of the variability (Mosier et al., 1983; Robertson and Tiedje, 1984). However, by using parameters which indirectly could control denitrification, i.e. soil texture and soil drainage, Groffmann and Tiedje (1989) were able to explain 86% in the annual loss caused by denitrification. This lead to the observation that parameters controlled at the landscape scale are more effective predictors for denitrification activity than small scale soil and environmental factors (Groffman, 1991).

In a previous study, denitrification activity after the application of fertilizer-N and the first irrigation event of the season was estimated using the large landscape scale approach (Pennock et al., 1992). From this study the conclusion was drawn that (1) different landform elements showed different rates of denitrification and, (2) that landscape-scale pattern of denitrification are controlled by individual soil processes which in turn are controlled by more fundamental hydrological and pedological processes.

In the aforementioned study, denitrification activity was estimated only once at the onset of the growing season and no further sampling was carried out to confirm if the pattern of denitrification activity in the various identified landform elements remained similar throughout the growing season. This lead to the objective of the current reported study to establish if similar landform elements would consistently show higher denitrification activity throughout the growing season.

MATERIALS AND METHODS

Field description and sampling

A site was selected in 1991 in the Dark Brown soil zone near Blaine Lake, Saskatchewan, Canada. A 140-m by 140-m area (1.96 ha) was selected in a representative section of a field that would be cropped to pea (*Pisum sativum* L.). A grid composed of 144 sample sites separated by a spacing of 10 m was placed and surveyed. Six landform elements were determined: upper level, divergent shoulders, convergent shoulders, divergent footslopes, convergent footslopes and lower level. From each landform elements, ten sites were selected and sampled throughout the growing season for denitrification using the acetylene-blockage technique (Yoshinari et al., 1977). In addition to denitrification activity, from every sampling point at every sampling period, denitrification activity, soil moisture content, NH_4^+, NO_3^-, water soluble organic content, and soil respiration were determined as described by Pennock et al. (1992) with the following modifications. At each sampling point eight soil cores (10 cm x 4 cm i.d. aluminum cylinders) were taken: four soil cores were placed in one incubation vessel and the soil incubated in the presence of acetylene (7.5% v/v). After an incubation period for 24 h, the gas phase was analyzed for N_2O by gas chromatographic determination of N_2O carried out as described by Hynes et al., (1985). Soil of the 4 soil cores taken from each sampling point were combined for further soil analysis. The concentration of NH_4^+ and $NO_3^- + NO_2^-$ in soil extracts was determined by steam distillation after the addition of MgO for NH_4^+ determination followed by the addition of Devarda alloy for the determination of $NO_3^- + NO_2^-$ (Keeney and Nelson, 1982).

Samples at the selected 60 points in the sampling grid were taken seven times throughout the growing season: before the preparation of the field for seeding of pea (May 8), following a precipitation event (May 10), after the application of N fertilizer (20 kg N as $(NH_4)H_2PO_4$, May 28), following a precipitation event (June 4), at flowering (June 27), podfill (Aug. 6) and at the onset of frost (Oct. 13). At every sampling period, analysis were carried out as described above.

To establish the duration of denitrification activity after the occurrence of precipitation event, the production of N_2O in soil cores incubated with acetylene was followed before and after the application of 50 mm of water on 15 selected areas, representing all the six landform elements. Samples were taken immediately before and after the application of water and continued for 30 h at 6 h intervals after the application of water. Similar procedures for the determination of N_2O in the soil cores as described previously was followed.

Statistical analysis

Exploratory data analysis as described by Pennock et al. (1992) was used in the first stage of statistical analysis. The frequency distribution of denitrification activity did not allow the use of parametric statistics without transformation of the data. However, instead of transforming the data, non parametric statistics were used and the significance of differences between soil parameters at the various landform elements assessed using Kruskal-Wallis one-way analysis of variance by ranks. Rankings were corrected for ties (Siegel and Castellan, 1988). The significance between denitrification activity and the various soil parameters was assessed by Spearman correlation, appropriate for not normally distributed data.

RESULTS AND DISCUSSION

Site description

The field site is located on a gently-sloping surface underlain by silty glacio-lacustrine deposits. A major depression was located in the N corner of the field site. This lower lying area or slough had not been cultivated earlier but was brought into cultivation in 1991. No standing water after a precipitation event, however, occurred in the major depression throughout the growing season.

Six landform elements occupying the following percentage of the total area were identified; upper level, 14.2% ; divergent shoulders, 19.9%; convergent shoulders,12.8%; divergent footslopes, 13.1%; convergent footslopes, 17.9%; and lower level, 22.1%.

Temporal denitrification activity

Denitrification activity varied greatly during the year (Table 1). Activity was low before the field was prepared for seeding in the spring but increased sharply two days later following a precipitation event. Precipitation and irrigation events increase total anaerobiosis in the soil and this lead often to an increase in denitrification activity (Craswall, 1978, Smith and Tiedje, 1979).

Whereas the application of fertilizer-N by itself did not enhance denitrification, the highest denitrification activity was observed the first precipitation event following the application of fertilizer-N (May 28) with an overall field average rate of denitrification of 386 g N ha^{-1} day^{-1}. High rates of denitrification were also observed toward the end of the month of June when the crop was fully established following a precipitation event that occurred within 24 hours of sampling. Temperature has a marked effect on denitrification activity (Firestone, 1982) and the increase in denitrification activity for the two June sampling periods can be partially explained by the increase in soil temperature. Toward the end of the growing season and before the onset of the fall frost and the concurrent low soil temperature, denitrification activity became almost nil.

Table 1. Temporal denitrification activity and ambient evolution of N_2O.

Sampling period		Mean	Median g ha^{-1} day^{-1}	Min.	Max.	CV %	Skewness	# extreme outliers
Before land preparation 8-May	Denitrification	32	3	0	448	232	3.80	6
	Ambient N_2O	11	1	0	199	303	4.55	5
Following precipitation 10-May	Denitrification	138	61	0	939	127	2.33	3
	Ambient N_2O	48	24	0	426	155	3.24	5
Application of N-fertilizer 28-May	Denitrification	26	1	0	743	382	6.50	10
	Ambient N_2O	12	2	0	181	221	4.57	7
Precipitation following seeding 4-Jun	Denitrification	386	177	0	1850	115	1.31	0
	Ambient N_2O	217	99	1	1217	134	1.99	4
Precipitation at early flowering 27-Jun	Denitrification	276	147	3	2267	142	2.94	3
	Ambient N_2O	127	45	2	1107	174	2.72	9
Pod fill 6-Aug	Denitrification	3	0	0	27	218	2.68	9
	Ambient N_2O	4	0	0	145	475	6.69	7
Onset of frost 13-Oct	Denitrification	0.2	0	0	2	212	2.20	10
	Ambient N_2O	0.2	0	0	5	339	5.40	11

Log transformation of denitrification activity failed of the various sampling periods often failed to approximate a normal distribution. Although a similar results was observed in an earlier study (Pennock et al., 1992), denitrification activity observed under field conditions is usually log normally distributed (Parkin et al., 1985). The often highly skewed frequency distribution lead to corresponding large C.V's (Table 1) which showed seasonal average of 204 %. Similar large C.V.'s were observed in other field studies and appear to be inherent to those *in situ* processes (Folorunso and Rolston, 1984). In this study, the frequency distribution of denitrification activity was less skewed when the area was sampled following a precipitation event (Table 1). After such an event the intensity of denitrification increased substantially and the number of sampling points with no or low activity became small or even disappeared completely (Table 1). Denitrification activity is often controlled by the concentrations of NO_3^-, O_2 (related to soil moisture content), soluble organic carbon, and the presence of denitrifiers. The average concentration of NO_3^- in the soil cores following a precipitation event ranged from 1.7 to 10.4 $\mu g\ g^{-1}$ soil

(Table 2). In soils, denitrification is often limited by the diffusion of NO_3^- to the denitrifying sites and the reaction follows a first-order pattern at lower NO_3^- concentrations (Knowles, 1982). A significant Spearman correlation coefficient ($P<0.01$) was observed between denitrification activity and the concentration of NO_3^- at the fourth and fifth sampling period when the highest denitrification activities were observed (data not shown). Although the concentration of NO_3^- were similar at the other sampling periods, sufficient NO_3^- was present to sustain low rates of denitrification activity that NO_3^- did not become limited. This suggests that from a particular concentration of NO_3^- *per se* it would difficult to determine if this concentration would be the limiting step for denitrification but that it more the overall potential rate of denitrification that determines if a particular concentration of NO_3^- will limit the reaction. Or, under conditions with low potential denitrification, low levels of N may not limit the reaction whereas under conditions with high potential denitrification, even much higher levels of NO_3^- may not be sufficient to sustain denitrification.

Table 2. Mean and standard error for soil variables during the season.

Sampling period	Moisture %	NO_3^- $\mu g\ g^{-1}$	NH_4^+ $\mu g\ g^{-1}$	Respiration $\mu g\ Cg^{-1}\ d^{-1}$	Soluble organic carbon $\mu g\ g^{-1}$
Before land preparation 8-May	24.2+0.4	4.6+2.9	1.0+0.8	14.6+0.8	49.1+3.2
Following precipitation 10-May	29.9+0.5	1.7+0.5	2.4+0.2	15.6+0.7	50.5+3.4
Application of N-fertilizer 28-May	13.7+0.4	10.4+0.6	5.3+0.7	23.6+1.1	47.9+2.7
Precipitation following seeding 4-Jun	28.9+0.4	9.1+1.0	2.7+0.4	25.4+1.0	56.2+3.7
Precipitation at early flowering 27-Jun	25.6+0.6	8.1+0.9	4.4+0.3	33.7+1.0	60.8+3.0
Pod fill 6-Aug	10.2+0.5	2.1+0.1	2.8+0.1	21.9+2.2	85.1+4.3
Onset of frost 13-Oct	10.9+0.2	8.3+0.6	2.7+0.1	6.2+1.1	51.0+2.0

Water soluble organic carbons ranged from 49.1 $\mu g\ g^{-1}$ soil in the spring to a maximum of 85.1 $\mu g\ g^{-1}$ soil for the August sampling (Table 2). Burton and Beauchamp (1985) found that the concentration of soluble organic carbon required to promote denitrification activity was in the range of 60 to 80 $\mu g\ g^{-1}$ soil. In fact, the concentration of water soluble carbons never showed a correlation with denitrification activity.

The large increase in denitrification activity following a precipitation event, indicates that soil moisture was a critical factor controlling denitrification. This was followed by the concentration of NO_3^- when high denitrification activity occurred in conjunction with an increase in soil temperature. By partially eliminating those controlling parameters on denitrification activity (a condition that occurred after the application of fertilizer and the occurrence of an precipitation event on June 4) the frequency distribution of denitrification activity became less skewed. A similar observation was made by Christensen et al. (1991) who found that the frequency distribution of denitrification rates was not or became less skewed under conditions of high activity: in flooded soil, after the additions of glucose and after the decomposition of plant litter. Similar observations were observed in poorly drained loam and clay forest soils which showed high rates of denitrification and a non-skewed frequency distribution in the spring whereas well drained soils showed low activity and a log-normal distribution distribution with coefficients of variation larger than 100 throughout the season (Groffman and Tiedje, 1989). By eliminating rate limiting steps in the denitrification reaction, the frequency distribution becomes normally distributed. By eliminating those various steps, however, the overall denitrification activity will increase to maximum values.

Denitrification by landform element

Throughout the season, the highest denitrification activity was observed on the two footslopes and the lower level landform elements. In contrast, the lowest activity was found on the shoulders and upper landform elements (Table 3). Whereas the average denitrification activity at the divergent shoulder on the May 8 sampling amounted to only 1 g ha^{-1} day^{-1}, at the divergent footslope the average denitrification activity was estimated at 93 g ha^{-1} day^{-1}. A similar large difference between denitrification at those two landform elements was found for the May 28 sampling after the application of fertilizer. Differences between the various landform elements, however, became less obvious after the occurrence of a precipitation event (Table 3). The lower rates of denitrification on the divergent shoulders and higher rates of activity on the footslopes remained consistent throughout the year, regardless of the magnitude of denitrification activity, and a clear, consistent seasonal pattern at the various landform elements for denitrification activity was present. Using the Kruskal-Wallis one way analysis of variance, the first five sampling periods showed significant differences in denitrification activity among the various identified landform elements. After ranking the various landform elements by denitrification activity and followed by determination of the Z-value ($\alpha = 0.20$), the divergent shoulder showed significantly lower denitrification activity than the divergent footslopes (Table 3). The distinct different activities for denitrification associated with the various landform elements indicates a topographical control on the parameters regulating denitrification (Pennock et al., 1992). Topography by itself will not be the controlling factor for denitrification activity but rather soil texture and water flow who are controlled by hydrologic and pedologic processes which, in turn, are regulated by topography. Groffman and Tiedje (1989) found a strong relationship between denitrification and soil texture and drainage across a forested landscape in Michigan. Both soil texture and drainage are controlled, however, indirectly by topography. Finer soil particles, in particular clay particles, will be transported, mainly through hydrological processes, from the upper parts of the landscape to the the lower areas and streams. The authors were able to explain 86 % of the variability in the annual loss to denitrification by using soil texture and drainage in a multiple regression model.

Whereas the June 27 sampling showed the second highest seasonal rate of denitrification, the probability of significant differences in denitrification between the six landform elements was only 0.18. A possible explanation for a lower significant relationship between denitrification and landform elements might have been caused by the depletion of O_2 by root and microbial activity. Root and microbial respiration reduces the

Table 3. Seasonal denitrification and ranking at the various landform elements.

Landform element	Sampling date: 8 May		10 May		28 May		4 June		27 June		6 August		13 October	
	Mean g N ha^{-1} day^{-1}	Ranking	Mean g N ha^{-1} day^{-1}	Ranking	Mean g N ha^{-1} day^{-1}	Ranking	Mean g N ha^{-1} day^{-1}	Ranking	Mean g N ha^{-1} day^{-1}	Ranking	Mean g N ha^{-1} day^{-1}	Ranking	Mean g N ha^{-1} day^{-1}	Ranking
Upper level	19	28	165	34	6	26	214	25	85	20	2	30	0.2	30
Divergent shoulder	1	18	34	16	2	22	135	14	107	23	1	21	0.2	21
Convergent shoulder	12	25	90	26	14	25	302	28	359	33	3	25	0.0	25
Divergent footslope	93	39	236	37	95	39	617	41	330	34	4	31	0.2	25
Convergent footslope	40	38	161	35	39	39	625	35	472	31	8	36	0.2	32
Lower level	25	30	130	32	7	27	416	34	328	32	1	27	0.2	27
Probability		0.06		0.09		0.08		<0.01		0.18		ns		ns
Z-value (x = 0.10) (one-tailed)		18		18		18		18		18		-		-

Table 4. Denitrification of the various landform elements in the spring and following a precipitation event.

Landform element	Percent of area	Spring rate	Following precipitation	Rate corrected for landform element per cycli: Spring	Precipitation	Spring	Precipitation
		g N ha-1 day-1		g N/landform element/cycle		% of total	
Divergent schoulders	19.9	1	135	0	25	1	7
Convergent shoulders	12.8	12	302	1	36	5	10
Divergent footslopes	13.1	93	617	11	76	42	21
Convergent footslopes	17.9	40	625	7	105	24	29
Upper level	14.2	19	214	3	28	9	8
Lower level	22.1	25	416	5	86	19	24

O_2 concentration in the soil although the consumption of O_2 by microbial respiration is considered to be of more importance than root respiration (Barber and Martin, 1976). Plants can have a strong effect on denitrification activity because root exudates and decomposing plant parts serve as a ready available C source for denitrifying bacteria and promotes microbial respiration (Bakken, 1988; Klemendtsson et al., 1987). Others, however, were not able to find an effect of plants on denitrification (Haider et al., 1985). Oxygen mainly enters the soil through diffusion but this process is limited by the presence of soil water caused by the larger diffusion coefficient for O_2 in air-filled pores than for water-filled pores (Smith, 1977). The occurrence of a precipitation event on June 26 and the seasonal increase in soil temperature would led to an increase in soil microbial activity. In fact, total soil respiration for the June 27 sampling period was the highest measured for the entire season (Table 2). In addition, at this time of the growing season the crop was fully established and an increased root respiration activity would further decrease the soil O_2 concentration and stimulate denitrification activity. Other parameters controlling denitrification activity, therefore, would become less dominant.

An evaluation of the overall ranking for denitrification activity of one particular sampling point revealed that throughout the growing season the ranking for denitrification for the 60 sampling points at one particular sampling period was often highly significantly correlated with the ranking for denitrification activity for the other sampling periods. Because of the low levels of denitrification activity toward the end of the season, the last two sampling periods were not considered. A consistent ranking of a particular sampling point for denitrification activity throughout the season reconfirms the continuous presence of the controlling parameters for denitrification at a sampling point observed earlier at the landscape scale. Because the ranking of the individual sampling points is independent of the overall intensity of denitrification in the landscape, the inherent soil properties of the soil such as texture, would have a regulatory control over the intensity of denitrification.

Denitrification activity following precipitation

Following the application of water at 15 locations in the landscape representing the various landform elements, a sharp increase in denitrification activity was observed in soil samples taken within one h after wetting the soil. Independent of the location in the landscape, maximum values for denitrification activities were observed within 4 h after the application of water and the activity declined within four hours after the application of water. Denitrification reached minimum values at the end of the time course experiment, 30 h after the onset of the experiment. No significant differences among the various landform elements between the percentage of maximum denitrification activity and the time after the application of water were detected. An absence of a lag period in denitrification activity was observed in a Yolo loam soil after application of water (Rolston et al., 1982). Others found that maximum denitrification in a sandy soil occurred immediately after the application of water but that for a clay loam soil it required 8-12 h before maximum activity was observed (Sextone et al, 1985). Denitrification reached pre-irrigation levels within 12 h and 48 h for the sand and clay loam soil, respectively. The difference in lag period is due to the water infiltration rate which is smaller for a clay soil than a sandy soil. Furthermore, the water holding capacity is larger for a clay soil than a sandy soil. Therefore, it would require a longer period before anaerobiosis is established in a clay soil but the anaerobiosis will last longer (Sextone et al., 1985).

The duration of denitrification activity following a precipitation event was estimated from the area under the relationship of the time after the application of water and the percentage of maximum activity. The estimated landscape scale seasonal denitrification activity following a precipitation event was 357 g N ha^{-1} $event^{-1}$ (Table 4). The majority of the activity occurred on the footslopes with only a minor contribution form the shoulders land form elements. For a similar period of time in the spring after a period of thaw, the losses of N for a similar period of time amounted to 27 g N ha^{-1} of which the majority was lost from the two foot slopes landform elements with only small looses from the shoulder landform elements.

CONCLUSIONS

Seasonal denitrification activity in a landscape is controlled by a range of soil and environmental factors. Whereas under low soil temperature and moisture content, low rates of denitrification were observed, the application of fertilizer-N, concurrent with a precipitation event and an elevated soil temperature increased the emission of N_2O to a seasonal maximum. A consistent landscape scale pattern throughout the season for both denitrification activity was observed: the highest level of N_2O production on the footslopes, the lowest activity on the shoulder landform elements. A similar seasonal pattern for the ranking of denitrification activity for individual sampling points was observed. The seasonal pattern of denitrification at the various landform elements and the ranking of the individual sampling points was independent of the magnitude of the activity. This confirms an earlier observation that denitrification activity was influenced strongly by topography which, in turn, controls more fundamental hydrological and pedological processes and their distribution in a landscape (Pennock et al., 1992).

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